REMARKS

Objections to claims 2, 33, and 35 have been corrected. The terms "phosphothiorate" and "phosphothioate" are used interchangeably in the biochemistry literature by persons of ordinary skill in the art. Although the applicant used the term "phosphothiorate" in the original application, the term was amended to "phosphothioate" on all occasions per the Examiner's request.

Amendments to claims 1, 28, 31, and 32 are supported on page 10, line 9 of the original application.

The applicant respectfully disagrees with the rejection of claims 1-3, 5-10, and 27 under 35 USC §112, second paragraph, for being indefinite and failing to particularly point out and distinctly claim the subject matter. The application clearly and distinctly defines a transcription factor decoy with a shear stress response element (SSRE) sequence as described in SEQ ID NO: 1 and Figure 4. Examples of common SSRE's are described in Resnick *et al.* FASEB J. 9:874-82 (7/95), incorporated by reference. The application specifically states on page 9, line 35, that "Representatives of shear stress response elements include GAGACC and GGTCTC." These elements are clearly defined in the art as short nucleotide sequences present in promoters and regulatory regions of many genes. SSRE's, as used in the context of this application, have been previously defined. Examples of shear stress response elements are shown by persons of ordinary skill in the art in Khachigian, *et al.* J. Clin. Invest. 96:1169-75 (8/95) "A 6-bp core element (5'-GAGACC-3'), defined previously as a shear-stress response element is present in the promoters of many genes..." and Nagel, *et al.* J. Clin. Invest. 94:885-91 (8/94) "We have recently identified a cis-acting transcriptional regulatory element, the shear stress response element (SSRE), present in the promoters of several genes ..." Shear stress response elements have been clearly defined in the field and are used as defined in this application.

Claim 10 is rejected under 35 USC § 112 as containing new matter. Applicants respectfully disagree. Claim 10 does not contain new matter because the original application and claim were directed to concentrations "from about 10 nm to about 10 mm" and the amended application and claim are directed to concentrations "from about 10 nM to about 10 mM" (page 10, line 4) as would be understood by a person of ordinary skill in the art to encompass the same

molar concentration range. The abbreviations for nano- and milli-molar have been amended per the Examiner's request from "nm" to "nM" and "mm" to "mM". The measurement of concentration is present throughout the application in terms of "nm", "mm" and "mmol" (page 11, line 29) as would be understood by a person of ordinary skill in the art. There is no confusion to persons of ordinary skill in the art between measures of distance "nm" or "nanometer", measures of time "rpm" or "revolutions per minute", and measures of concentration "mm" and "millimolar" for example. As used in this application and in the parent U. S. Patent 6,730,498, "mm" in the context of concentration represents "millimolar".

Applicants respectfully disagree with the rejection of claims 1-3, 5-10, 27, 28, 31-35 and 37, under 35 USC §112, first paragraph, as failing to comply with the written description requirement. The current amendments replace the term "encodes" with the term "comprising". As defined, transcription factor decoys are supported by the specification. The arguments made above demonstrate adequate written description for one of ordinary skill in the art to identify an SSRE.

Examples 11 and 15 demonstrate the induction and inhibition of differentially expressed genes in the presence of SSRE's. Proximal tubule markers are monitored throughout the application in response to various stimuli. The current SSRE constructs specifically induce ICAM, MnSOD, and EPO as demonstrated in Fig. 4 and Table 5. The application specifically identifies representative genes as selected from the group consisting of megalin, cubulin, erythropoietin, and 1-α-hydroxylase (page 10, lines 1-2). Other genes are monitored throughout the application as representative of shear stress response genes. Many of these genes coordinate with MnSOD expression and thus are either directly or indirectly influenced by the presence of SSRE containing decoys. Multiple SSRE's were assayed in genetic screens using SSRE containing expression vectors. Examples of SSRE's and the associated genes are described in Resnick, Tuttle, Melendez, and Hirose, incorporated by reference. Thus, there is ample description in the specification and references to identify genes regulated by SSRE containing transcription factor decoys. The use of differential display and genetic discovery arrays, as described in example 8 and example 10, are known to one of ordinary skill in the art and can be used to monitor multiple genes simultaneously.

The amendments to the claims were made to expedite the prosecution of this patent. The applicants retain the right to pursue the full scope of any claims in subsequent patent applications.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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Respectfully submitted,

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Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells.

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Hemodynamic forces induce various functional changes in vascular endothelium, many of which reflect alterations in gene expression. We have recently identified a cis-acting transcriptional regulatory element, the shear stress response element (SSRE), present in the promoters of several genes, that may represent a common pathway by which biomechanical forces influence gene expression. In this study, we have examined the effect of shear stress on endothelial expression of three adhesion molecules: intercellular adhesion molecule-1 (ICAM-1), which contains the SSRE in its promoter, and E-selectin (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1), both of which lack the SSRE. Cultured human umbilical vein endothelial cells, subjected to a physiologically relevant range of laminar shear stresses (2.5-46 dyn/cm2) in a cone and plate apparatus for up to 48 h, showed time-dependent but force-independent increases in surface immunoreactive ICAM-1. Upregulated ICAM-1 expression was correlated with increased adhesion of the JY lymphocytic cell line. Northern blot analysis revealed increased ICAM-1 transcript as early as 2 h after the onset of shear stress. In contrast, E-selectin and vascular cell adhesion molecule-1 transcript and cell-surface protein were not upregulated at any time point examined. This selective regulation of adhesion molecule expression in vascular endothelium suggests that biomechanical forces, in addition to humoral stimuli, may contribute to differential endothelial gene expression and thus represent pathophysiologically relevant stimuli in inflammation and atherosclerosis.

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